Acid-catalysed rearrangement of the sand fly pheromone sobralene to verticillenes, consolidating its relationship inter alia to the taxanes and phomactins

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Abstract The sex-aggregation pheromone sobralene 1, produced by the sand fly Lutzomyia longipalpis, is isomerised to the verticillenes 2 and 5 in the presence of mild acid, thereby providing credence to the proposal that sobralene is a likely shunt metabolite of the taxadiene synthase cascade.

Key words pheromone, sand-fly, verticillenes, metabolite, taxadiene

Sobralene 1 is a novel macrocyclic diterpene hydrocarbon recently characterised as the sex-aggregation pheromone produced by populations of the sand fly Lutzomyia longipalpis from Sobral, Brazil.1,2 The sand fly species complex L. longipalpis is the main carrier of the Protist parasite Leishmania infantum, the causative agent of American visceral leishmaniasis (AVL), which is a potentially fatal human disease in South and Central America.3,4

Sobralene 1 is the cis (Z)-C8,C9 isomer of the trans (E)-C7,C8 diterpene hydrocarbon 2 known as “verticillene” which, for several decades, was proposed as a likely key intermediate in the biosynthesis of taxadiene 3,5 and later, of the phomactin family of platelet activating factor (PAF) antagonists , e.g. 4,6 However, although a number of alkene isomers of verticillene, e.g. 5, ent-5, 6, 7, ent-7, have been characterised from various plant and other sources,7-10 the particular 3,7,11-triene isomer 2 has not been found “free” in Nature.11 In recent years however, in targeted engineering studies of taxadiene synthase (TXS) , Brück et al 12 demonstrated that particular mutants of TXS give rise to the production of verticillene 2 together with its isomers 5 and 6.
It has been proposed that the 6,12-membered ring-fused system in sobralene 1 is derived in Nature from geranylgeranyl diphosphate (GGPP) 8 using a similar taxadiene synthase (TXS) cascade pathway to that leading to taxadiene 3.1 Thus, macrocyclisation of GGPP accompanied by transannulation first leads to the ring-fused carbocation intermediate 9 (Scheme 1). Extensive investigations have established that transfer of a proton from C11 to C7 in 9 leading to the corresponding C8 carbocation 10 is a key step in the biosynthesis of taxadiene. The carbocation 10 can assume a number of conformations, and quantum chemical calculations have reinforced an earlier proposal that a change in conformation of the carbocation 10 from 11 to 12, is necessary prior to its transannulation to 13 en route to taxadiene 3.11 However, instead of transannulation, stereoselective elimination of the proton from C9, could lead to sobralene 1 (Scheme 1).1

The interesting similarities in structure between the newly discovered sand fly pheromone sobralene 1, and the verticillenes 2, 5-7, taxadiene 3 and phomactatriene 4, prompted us to probe these relationships by undertaking a study of the rearrangement of sobralene under acid conditions. These studies are presented here.

Male sobral sand flies produce extremely minute quantities of pheromone secretions, and only at specific periods in their life cycle.13,14 For our studies, we had batches of secretions which had been collected from many thousand sand flies over several months during different periods between 2014 and 2017, and then stored in different solvents. Fortunately, in a fresh sample of insect secretion (ca. 100 μg) collected in hexane from approx. two thousand male Sobral-2S sand flies, during four months in 2017, the pheromone was shown to constitute >90% of the secretion when analysed by GC-MS (Figure 1 in ESI). Study of this sample by high field 1H and 13C NMR spectroscopy then permitted us to establish the structure 1 for the pheromone.1

We also had a larger sample of secretion (ca. 1 mg) collected from ca. 40,000 sand flies during 2014 but which had been stored in deuterochloroform (i.e. mildly acidic conditions) for over 3 years. GC-MS analysis (Figure 2 in ESI) showed that this secretion contained five main constituents all of which had the same molecular ion at m/z 272, corresponding to C20H32. One of these compounds was present to the extent of ca. 36% (Rf 27.87) and a minor component (Rf 27.08, ca. 12%) corresponded to sobralene 1 from comparison of its GC-MS data with those of an authentic sample.

Examination of the EI mass spectrometry fragmentation data from the GC-MS analysis, alongside a library search on the NIST mass spectrometry database revealed that the fragmentation patterns for the main components in the insect secretion were all similar for isomers of the carbon backbone and substitution pattern observed for a "verticillene" hydrocarbon framework, cf. 5. With this foresight we then examined the proton and carbon NMR spectra of the secretion. The major component in the secretion showed 1H NMR data, i.e. δ 4.72 (dd, J 9.8, 1.0) and δ 5.19 (dd, J 12.0, 1.0) ppm which were diagnostic for the olefinic proton signals at C3 and at C7 respectively in the verticillene isomer 2 (Figure 3a in ESI). Likewise, other proton signals at δ 5.40 (brs), δ 5.28 (d, J 12.0) and δ 4.82 (d, J 12.0) in the secretion were superimposable on the C13, C3 and C7 olefinic proton resonances respectively in a synthetic sample of the isomeric verticillene 5 (Figure 3b in ESI). In addition, inspection and comparison of the data between δ 120 ppm and δ 140 ppm in the 13C NMR spectrum of the secretion showed correlations with those found in authentic samples of the isomeric verticillenes 2 and 5 (Figures 4a and 4b in ESI). Thus, the sample showed olefinic carbon resonances corresponding to verticillene 2 at δ 136.0 (s), 132.8 (s), 131.5 (s), 128.5 (d), 126.7 (d) and 126.3 (s) ppm, and to the endo verticillene 5 at δ 136.0 (s), 133.1 (s), 132.8 (s), 130.0 (d), 124.9 (d) and 121.8 (d) ppm (Figures 4a and 4b in ESI).

At this point in our studies we presumed that most of the sobralene 1 that had been present originally in the aforementioned decomposed secretion from the sand fly had undergone rearrangement via a sequence of proton exchanges leading to the isomeric verticillenes 2 and 5 during prolonged storage in the mildly acidic deuterochloroform solution (cf. Scheme 2). To give credence to this supposition we carried out a controlled experiment of the isomerisation of a fresh sample of insect secretion containing >90% sobralene collected in hexane solvent, in the presence of mild acid. Thus, after transfer from hexane to deuterochloroform a solution containing ca. 50 μg of sobralene (collected from ca. 1000 sand flies), was treated portion wise with a solution of 4N HCl in
dioxane (1 µL through to 5µL) at room temperature, and the disappearance of sobralene was monitored by GC-MS analysis of samples taken at regular intervals (see Figures 5a, 5b, and 5c in ESI). When the amount of sobralene, as measured by peak area in GC-MS analysis, had diminished to approximately 15-20% of the total products having the same molecular ion m/z 272 (C_{20}H_{32}) in mass spectrometry analysis, the solution was neutralised by passing it through a plug of anhydrous K_{2}CO_{3} located in a micropipette. The filtrate was then gently evaporated to dryness in vacuo and the residue was taken up in hexadeuterobenzene for further analysis by gas chromatography and NMR spectroscopy. GC-MS analysis showed the presence of one major product (>50%) and several minor products with m/z 272, in addition to a small amount of sobralene (<15%) in the residue (Figure 5c in ESI). With the very small amount of material available, data between δ 6.0 - 10.0 and δ 0.5 - 3.50 ppm in the proton NMR spectrum of the product resulting from the acid treatment of sobralene were largely meaningless (Figure 6 in ESI). However, analysis of the corresponding olefinic proton region between δ 4.75 - 5.5 ppm in the NMR spectrum showed that the major product in the sample was the verticillene isomer 2. This structure followed from analysis and comparison of its olefinic proton NMR data with those for synthetic verticillene and comparisons of its MS data (Figure 7 in ESI). Therefore, investigation of the acid-catalysed isomerisation of sobralene 1 into the verticillene 2 occurring in a straightforward manner via protonation of the cis (Z)-C8,C9 alkene bond in 1 followed by loss of the α-orientated proton at C7 in the resulting C8 carbocation intermediate 14 (cf. Scheme 1) leading to the trans (E)-C7,C8 alkene bond in 2 (Scheme 2). We propose that the endo (C12-C13) verticillene isomer 5 identified in the earlier described acid treatment of pheromone secretion is derived from the same carbocation intermediate 14 following initial transfer of the proton at C7 to C11 leading to the C18 carbocation 15 (cf. 9, Scheme 1), and then loss of a proton at C13 (Scheme 2). 17

The alkene isomers 5 and 7 of verticillene 2 were first reported in 1964 as products resulting from the dehydration of the natural product (+)-verticillol 16, isolated from the wood of Sciadopitys verticillata 15,16. Later, in 2005, the same verticillenes 5 and 7 were isolated together with the 4-exo
verticillene isomer 6 from plants of the genus Bursera endemic to Mexico. At a similar time, during investigations of the treatment of verticillol 16 with boron trifluoride etherate at -78 °C Oikawa et al.10 identified the interesting phomactatriene 17 in addition to the verticillenes 5 and 7 amongst the products. Indeed the same BF3-catalysed reaction with verticillol at -30 °C led to 17 as the major product. In complementary studies Oikawa and his colleagues later identified the isomeric verticillenes 5 and 7 together with the phomactatriene 17 in mycelium extracts of the marine fungus Phoma sp. The phomactatriene 17 has its origins in the same C12 carbocation intermediate 15 proposed as an intermediate in the biosynthesis of both sobralene 1 and taxadiene 3 from GGPP 8 (cf. Scheme 1). Thus, a sequence of carboxylation initiated 1,2 hydrogen and 1,2-methyl group shifts in 15 first leads to the rearranged cyclohexane carbocation 18 which, following elimination of a proton at C1, produces the phomactatriene 17 (Scheme 3).

As a corollary to our studies we undertook an independent study of the rearrangement of verticillol using different acid conditions to determine whether or not phomactatriene structures might be amongst the products of the rearrangement of sobralene 1 in acid. For these studies we were fortunate to obtain a sample of 12-epi verticillol 19 which had only recently been isolated from Bursera microphylla by Marcotullio et al.11 Treatment of a solution of 12-epi verticillol 19 in CDCl3 with 4N HCl in dioxan at room temperature, resulted in its complete conversion into endo-verticillene 5, within 45 min, in essentially quantitative yield.19 Similarly, treatment of 19 with TFA in benzene at 60 °C also led to the endo-verticillene in 80% yield, albeit more slowly (i.e. ca 5 h). By contrast however, treatment of a solution of 12-epi verticillol 19 with p-TSA:H2O in benzene at 80 °C for 20 mins resulted in a facile rearrangement via the carbocation intermediates 15 and 18 leading to the formation of the interesting iso-phomactatriene 20 as the only isolable product in 71% yield.20 iso-Phomactatriene 20 is a positional isomer of the known phomactatriene 17 where the alkene bonds at C2-4 and C7-8 in 17 have migrated to C3-4 and C8-9 respectively in 20 (Scheme 4). Interestingly, the phomactetriene isomer 20 was earlier reported amongst the products of treatment of (+)-verticillol 16, isolated from Sciadopits verticillate, with lanthanum triflate, and the same authors also demonstrated that 17 could be isomerised to 20 in low yield following treatment with BF3 etherate at -20 °C. Notwithstanding these interesting observations, however, we were not able to secure any evidence for the presence of phomactetriene-like structures amongst the products of acid-catalysed rearrangement of the pheromone sobralene 1 produced by the sand fly Lutzomyia longipalpis.

In summary we have demonstrated that the major products which result from treatment of the sex aggregation pheromone sobralene 1 of the sand fly L. longipalpis from Sobral, Brazil with dilute acid are the isomeric verticillenes 2 and 5. The results reinforce our earlier suggestion of the close relationship between sobralene and verticillenes in Nature and, inter alia, the taxane 3 and phomactin 4 families of natural products. Interestingly, the processes which result in the formation of the verticillenes 2 and 5, sobralene 1, and taxadiene 3 (also phomactatriene 17) in Nature share the common C8 verticillyl carbocation intermediate 10 (cf. 14). This observation illustrates that very subtle changes in the conformation of 10 present several opportunities for its subsequent chemistry in vivo.

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Supporting Information

YES (this text will be updated with links prior to publication)

Primary Data

NO (this text will be deleted prior to publication)

References and Notes


(17) Further studies are underway to elucidate whether a one-step transfer of the proton at C7 to C11 occurs or if discrete steps are involved.


(19) Trifluoroacetic acid (1.0 µL, 0.0130 mmol) was added to a solution of 12-epi verticillol 19 (1.20 mg, 0.00413 mmol) in C6D6 (0.75 mL) and the resulting solution was heated at 60 °C for 3 hours. A further portion of trifluoroacetic acid (2.0 µL, 0.0260 mmol) was added, and the solution was heated for another 2 hours. The solution was cooled to room temperature and evaporated to dryness. The residue was purified by silica gel chromatography, eluting with pentane to afford endo-verticillene (0.90 mg, 80%) as a pale yellow oil. HR-EI-MS (C20H32+, M+) requires 272.2499; HR-EI-MS (C45H39O2+, M-C4H9O) requires 361.2499; HR-EI-MS (C20H32+, M+) requires 272.2499.

(20) pTSA.H2O (1.5 mg, 0.00517 mmol) was added to a solution of 12-epi verticillol 19 (1.5 mg, 0.00517 mmol) in C6D6 (0.75 mL) and the resulting solution was heated at 80 °C for 20 minutes. The solution was cooled to room temperature, then passed through a short plug of silica gel chromatography, eluting with Et2O (1 mL) and evaporated. The residue was purified by silica gel chromatography, eluting with pentane to give the iso-phomactatriene (1.8 mg, 71%) as a pale yellow oil; 1H-NMR (500 MHz, C6D6) 5.02 (1H, m, H9), 4.77 (1H, d, J = 10.3 Hz, H5), 2.60 (1H, dt, J = 2.4, 13.2 Hz, H6a), 2.39 (1H, m, H1), 1.97-1.90 (2H, m, H5b and H6b), 1.90-1.81 (2H, m, H2b and H3b), 1.52 (3H, s, H18), 1.45 (3H, s, H12), 1.42 (1H, m, H11), 1.33-1.29 (2H, m, H2a), 0.88 (3H, s, H16), 0.81 (3H, s, H17). 13C-NMR (125 MHz, C6D6) 133.3 (C15), 132.8 (C4), 130.4 (C7), 125.1 (C3), 122.2 (C13), 42.9 (C11), 41.3 (C5), 40.0 (C9), 38.0 (C1), 36.0 (C15), 34.5 (C2), 31.2 (C14), 27.2 (C7), 23.4 (C16), 23.4 (C18), 21.9 (C10), 15.3 (C9), 15.3 (C20). GCMS (C20H35+, M+) requires 272.2499; HR-EI-m/z found 272.2551.